Cyclopropylmethyl Compounds as Probes of the Mechanism of Hydrogen Transfer by NAD(H) and Alcohol Dehydrogenase

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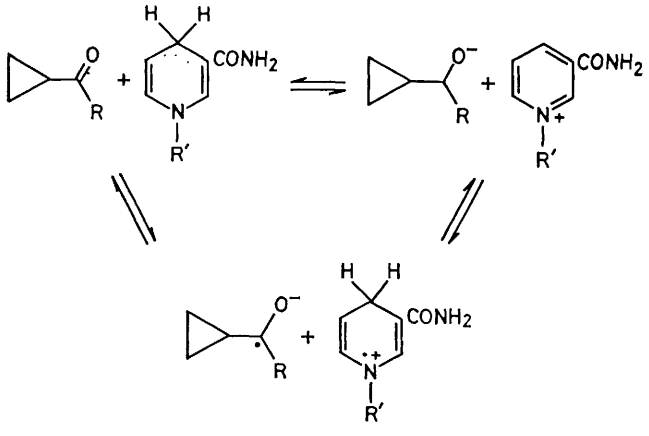
α-Hydroxyalkylcyclopropanes are oxidised by nicotinamide-dependent horse liver alcohol dehydrogenase without cleavage of the 3-membered ring, implying that radical intermediates are improbable in these reactions.

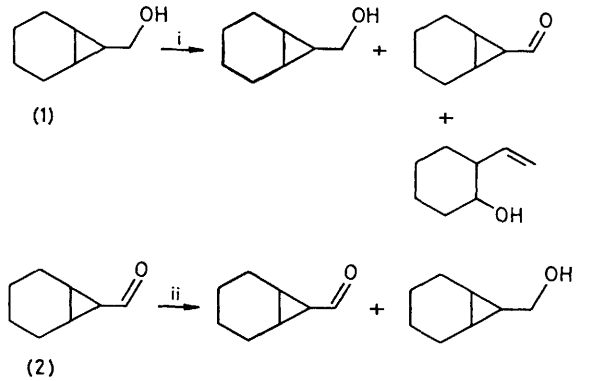
The question of the mechanism of hydrogen transfer by nicotinamide coenzymes and dehydrogenases and by their non-enzymic model systems has been extensively debated.[1] Although it has generally been accepted that the enzyme-catalysed reactions proceed by hydride transfer, a series of reports has persistently argued for the intermediacy of radicals in model systems.[2] The relevance of model systems to their enzymic prototypes is always difficult to establish, and in this case, special care needs to be taken because the claims for radical intermediates in model reactions have been strongly criticised.[3] Nevertheless, one-electron oxidation of dihydropyridines is reasonable with suitable substrates[1,4] and it has been argued on theoretical grounds that the mechanism of dihydropyridine oxidation should be substrate-dependent.[5] It therefore seemed important to investigate dehydrogenase-catalysed reactions with a probe that minimises abstraction from the natural system. Cyclopropylmethyl compounds provide an opportunity to distinguish between radical and hydride pathways (Scheme 1) because the cyclopropylmethyl radical has been shown to undergo unimolecular ring opening with a rate constant of the order of 10^8 s^{-1} at 25 °C.[6] In contrast, solvolytic reactions of cyclopropylmethanol derivatives, which involve polar transition states, in common with the hydride mechanism, occur 10^{11} times more slowly with substantial retention of the cyclopropane ring.[7] Typical rate constants for hydrogen transfer by alcohol dehydrogenase have been estimated as between 32 and 150 s^{-1}.[8] Thus it would be expected that a cyclopropanemethanol on oxidation by alcohol dehydrogenase should preserve its three membered ring and the same should be the case for reduction of the corresponding carbonyl compound.

We have recently shown that *exo*-bicyclo[4.1.0]heptane-7-methanol (**1**) is a substrate for, and a latent inhibitor of, horse liver alcohol dehydrogenase.[9] This compound is well suited to the experiment outlined above since it can readily be accommodated by the enzyme's active site without conformational restriction about the ring $-CH_2OH$ bond as shown by correlation with the established requirements of the active site.[10] Kinetically, the similarity of this substrate (**1**) to ethanol can be demonstrated by comparing the Michaelis constants. K_M for (**1**) was found to be 1.5×10^{-4} mol l^{-1} compared with 4.1×10^{-4} mol l^{-1} for ethanol and the relative maximum velocity of oxidation of (**1**) was 60% of that of ethanol. To determine the result of oxidation/reduction by alcohol dehydrogenase, we submitted the alcohol (**1**) to preparative scale enzyme oxidation using catalytic quantities of NAD^+ recycled by FMN[11] (Scheme 2). Thus the alcohol (**1**) (0.04 M) was dissolved in pH 9 0.1 M phosphate buffer containing NAD^+ (0.04 M) and FMN (0.08 M) and incubated at room temperature for 3 days with horse liver alcohol dehydrogenase (Sigma, 15 mg added in 3 portions). The products were recovered by continuous extraction and characterised by g.l.c. on two columns (5% FFAP on Chromosorb W and 5% Apiezon on Chromosorb W) by comparison with synthetic standards and by ^1H n.m.r. spectroscopy. Unchanged alcohol (**1**), and corresponding aldehyde (**2**) were the major compounds recovered (7 : 3, *ca.* 80% recovery), and, notably, the cyclopropane ring was intact. In addition, a small proportion of 2-vinylcyclohexanol (*ca.* 10%), apparently an enzyme-catalysed solvolysis product of (**1**), was detected.[9] The reverse reaction starting from the aldehyde (**2**) was carried out similarly but using pH 7 0.1 M phosphate buffer and sodium dithionite as recycling agent. In this case, only the aldehyde (**2**) and the cyclopropanemethanol (**1**) were the products (1 : 2). Clearly, in both senses of reaction, the cyclopropane ring is not cleaved in the manner of a cyclopropylmethyl radical.

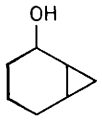
Before drawing conclusions concerning the mechanism of hydrogen transfer, we wished to confirm that the substrate chosen would undergo redox reactions *via* hydride and radical pathways typical of our expectations. The trimethylsilyl ether of (**1**) was oxidised by hydride abstraction using triphenylmethyl tetrafluoroborate in dichloromethane[12] and a quantitative yield of the aldehyde (**2**) was obtained. Radical reduction of the aldehyde (**2**) was demonstrated using tri-n-butyltin hydride[13] and azo-bisisobutyronitrile as initiator affording, after work up, cyclohexane-ethanol in 86% yield. A low conversion (5%) of aldehyde into this compound was also obtained by photolysis in isopropyl alcohol. These results confirm the reaction paths for radical and hydride mechanisms



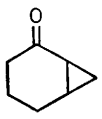
Scheme 1. R = H or alkyl.



Scheme 2. i, HLADH, NAD, FMN, pH 9, 3 d; ii, HLADH NAD, $Na_2S_2O_4$, pH 7 3 d.



(3)



(4)

suggested by precedent. It therefore seems improbable that a radical intermediate is involved in the horse liver alcohol dehydrogenase-mediated reaction of alcohol (**1**) and aldehyde (**2**).

In addition to primary alcohols and aldehydes, cyclic secondary alcohols and ketones are also good substrates for alcohol dehydrogenase. To extend the basis for our mechanistic conclusions we have examined bicyclo[4,1,0]heptan-2-ol (**3**) and the corresponding ketone (**4**). Davies[14] has already demonstrated, using e.s.r. spectroscopy, that the cyclopropylmethyl radical derived from the tri-n-butylstannyl ether of (**3**) undergoes ready ring opening and we were therefore confident that (**3**) was a good probe of the enzyme's mechanism. The trimethylsilyl ether of (**3**) also undergoes oxidation with triphenylmethyl tetrafluoroborate to yield the ketone (**4**). Preparative enzyme-catalysed oxidation of (**3**) under the same conditions as the oxidation of (**1**) led to a quantitative yield of the ketone (**4**). The reverse reaction was unsuccessful under normal reducing conditions (NADH, pH 7) because the rate of reduction of (**4**) to (**3**) is exceedingly slow and unchanged ketone was recovered. As far as could be ascertained, therefore, with the secondary alcohol/ketone system, ring opening does not occur either. The substrates that we have used, although demonstrably similar to the natural substrates, are nevertheless analogues and any conclusions drawn require extrapolations which we believe to be minimal in this case. With this in mind, we conclude that the cyclopropyl substrates provide no evidence for radical intermediates in typical reactions catalysed by horse liver alcohol dehydrogenase.

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